Homocysteine and Coronary Atherosclerosis

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Homocysteine is increasingly recognized as a risk factor for coronary artery disease. An understanding of its metabolism and of the importance of vitamins B₆ and B₁₂ and folate as well as enzyme levels in its regulation will aid the development of therapeutic strategies that, by lowering circulating concentrations, may also lower risk. Possible mechanisms by which elevated homocysteine levels lead to the development and progression of vascular disease include effects on platelets, clotting factors and endothelium. This review presents the clinical and basic scientific evidence supporting the risk and mechanisms of vascular disease associated with elevated homocysteine concentrations as well as the results of preliminary therapeutic trials.

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Homocysteine Metabolism

Homocysteine is an intermediate formed during the metabolism of methionine, an essential sulfur-containing amino acid supplied from dietary proteins. The recommended dietary allowance for methionine in U.S. adults is 0.9 g/day (1), and the estimated adult intake in this country is ~2 g/day. The first step in the metabolism of methionine (Fig. 1) is the formation of S-adenosylmethionine, which is an important methyl donor in many transmethylation reactions (2). S-Adenosylmethionine is demethylated to form S-adenosylhomocysteine, which is then hydrolyzed to adenosine and homocysteine. Homocysteine can then either enter the transsulfuration pathway or the remethylation cycle. Approximately 50% enters the transsulfuration pathway, where it is irreversibly combined with serine by the B₆-dependent enzyme cystathionine beta-synthase to form cystathionine. This is then metabolized to cysteine and alpha-ketobutyrate by gamma-cystathionase, another B₆-dependent enzyme. In situations of excess methionine, the transsulfuration pathway is favored by up-regulation of cystathionine beta-synthase and down-regulation of the remethylation pathway (3). The cysteine that is formed from homocysteine is ultimately converted to the sulfate and is excreted in the urine.

In the remethylation pathway, homocysteine is recycled to methionine by two different reactions. The first requires the presence of S-methyltetrahydrofolate-homocysteine methyltransferase (methionine synthase). Methylcobalamin and methyltetrahydrofolate serve as cofactor and cosubstrate for this enzyme. The remethylation pathway is favored during relative methionine deficiency, and this recycling and conservation of homocysteine ensures adequate methionine maintenance (2). A second pathway exists for homocysteine remethylation catalyzed by the enzyme betaine-homocysteine methyltransferase (2).

Homocysteine is thus an important reflection of the status of methionine metabolism, and its metabolic fate can be
Sulfate + H$_2$O

Urine

Figure 1. Methionine cycle: metabolic pathways of homocysteine metabolism. DMG = dimethylglycine; MTHF = methylenetetrahydrofolate; NADP$^+$ = nicotinamide adenine dinucleotide phosphate (oxidized form); NADPH = nicotinamide adenine dinucleotide phosphate (reduced form).

Influenced by alterations in the concentrations of folate, B$_6$, B$_12$, and activities of the various enzymes that participate in the transsulfuration or remethylation pathways. Normally, the intracellular concentration of homocysteine is kept within narrow bounds (4), and any increase in production (5) or reduction in metabolism (6) is met by export from cells. Conversely, if the formation of homocysteine is reduced, export from cells decreases (7). The concentration of homocysteine in blood is therefore an important reflection of its intracellular concentration and of the integrity of the various pathways responsible for its metabolism.

Circulating forms of homocysteine and determination. Approximately 80% of homocysteine in blood is protein bound by disulfide linkage (8). The remaining unbound homocysteine combines by oxidation either with itself to form the dimethyl homo cysteine or with cysteine to form the mixed disulfide cysteine-homocysteine. Only a small amount circulates as free homocysteine (8). When blood is drawn, free homocysteine becomes protein bound, even when samples are frozen immediately (9). In such samples, therefore, free homocysteine may be variable, but total homocysteine remains constant. However, total homocysteine may increase if whole blood is stored at room temperature; this may reflect the export of homocysteine from red blood cells (10).

Many early clinical studies of homocysteine used plasma mixed disulfide (11-14), homocysteine (12,14) or protein-bound homocysteine (15) as indicators of homocysteine status. In 1985, Refsum et al. (9) developed an assay for the determination of total homocysteine, including both protein-bound and free fractions; another assay for total homocysteine was developed at the Cleveland Clinic Foundation (16). Fasting samples have been widely used for the measurement of plasma homocysteine. Malinow et al. (17) reported similar values for fasting and postprandial samples, although small differences have been reported by others (18). However, for standard reference purposes, total fasting plasma homocysteine is recommended. Samples of blood should be placed on ice immediately on drawing and the cellular components removed within 2 to 3 h. Values in a normal population may vary slightly between laboratories but usually lie in the range of 5 to 15 μmol/liter in the fasting state (16,18). A level greater than this is often referred to as hyperhomocysteinemia.

Methionine loading test. In some subjects with impaired homocysteine metabolism, fasting concentrations may be normal. A methionine loading test may be used to expose this latent abnormality (4). Similar in principle to the glucose tolerance test, loading is performed by the administration of an oral dose of methionine, 0.10 g/kg body weight. Plasma homocysteine levels increase and may be measured 2, 4, 6 or 8 h later (12,13) (Fig. 2). There is some variability in the time at which peak values are seen: free homocysteine levels may peak at 2 to 3 h, whereas protein bound levels may peak later at 4 to 6 h (4). Recent studies of homocysteine in disease states have utilized fasting total homocysteine concentrations (19-22), with con-
elusions similar to those of earlier studies utilizing the methionine loading test (11–13). Given the cost and time required to perform the methionine loading study, a single fasting sample may be the most cost-effective test.

Factors Influencing Homocysteine Metabolism and Causes of Hyperhomocysteinemia

Plasma homocysteine concentrations are regulated by a number of enzymes, essential cofactors and the availability of the important cosubstrate methylenetetrahydrofolate. Predictably, the causes of hyperhomocysteinemia are multifactorial (Table 1).

Genetics. Homozygotes for classical homocystinuria have low or undetectable activity of cystathionine beta-synthase and a characteristic excessively elevated plasma homocysteine. Conversely, because the gene for cystathionine beta-synthase is located on chromosome 21 (4), increased activity of cystathionine beta-synthase might be expected in trisomy 21 Down syndrome. Decreased total plasma homocysteine was found in eight pediatric patients with Down syndrome (4). A strong genetic influence on plasma homocysteine concentration has also been seen in studies of normal subjects (23) and in patients with vascular disease (20,24,25). Inherited remethylation cycle abnormalities have been described, including derangements of methionine synthase caused by disorders of cobalamin metabolism (4,27–29). A number of disorders of methylenetetrahydrofolate reductase have also been described (4,30–36). In some of these, the precise genetic abnormalities have been identified (32–36). In all these situations, elevated homocysteine concentrations may be seen.

Age. Plasma homocysteine levels increase with age (4,15) for reasons that are obscure. Decreases in cofactor levels (37,38) or coexisting renal impairment often seen in older patients may be responsible, and age-dependent reductions in cystathionine beta-synthase activity may also play a part (39).

Gender. In general, men have higher plasma levels than women (15,16). Levels increase in both genders with age (15). After menopause, fasting homocysteine concentrations may increase (15,40) or remain unchanged (38). Although gender differences may be explained by the effect of sex hormones on homocysteine metabolism, they may be related to higher creatinine values (41) or the greater muscle mass of men than women (38).

Renal function. There is a positive correlation between fasting plasma homocysteine and serum creatinine (22,38), though the mechanism is unclear. Both renovascular atherosclerosis and prerenal factors may be important (22). In chronic renal failure, plasma homocysteine levels may be two to four times normal (42,43). These concentrations decrease after dialysis (43). Increases in homocysteine in renal failure may result from impaired metabolism rather than excretion (43).

Nutrition. Vitamin B<sub>12</sub>-folate. Homocysteine levels can be markedly elevated in deficiencies of the essential cofactor vitamin B<sub>12</sub> (44) or of the cosubstrate folate (45,46). Indeed, plasma homocysteine levels may be elevated even when serum B<sub>12</sub> and folate levels are in the low normal range (37,45,47). Negative correlations between serum folate and B<sub>12</sub> and homocysteine have been seen in studies of normal subjects (16,37,48,49).

Vitamin B<sub>6</sub>. There are conflicting results concerning the effects of vitamin B<sub>6</sub> deficiency on homocysteine in humans (48,50), although negative correlations similar to those seen with B<sub>12</sub> and folate have been observed in normal subjects (37).

Table 1. Factors Influencing Homocysteine Levels

<table>
<thead>
<tr>
<th>Cause</th>
<th>Description</th>
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<tbody>
<tr>
<td>I. Genetics</td>
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<tr>
<td>A. Transsulfuration</td>
<td>Abnormalities: diminished or absent cystathionine-beta-synthase activity (chromosome 21)</td>
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<td>abnormalities</td>
<td></td>
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<tr>
<td>B. Remethylation</td>
<td>Abnormal methylenetetrahydrofolate reductase (absent or thermolabile variant)</td>
</tr>
<tr>
<td>abnormalities</td>
<td></td>
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<tr>
<td>C. Abnormal methionine</td>
<td>Homocysteine increases with age</td>
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<tr>
<td>synthase</td>
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<tr>
<td>II. Age/gender</td>
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<tr>
<td>A. Homocysteine</td>
<td>Men &gt; age-matched women</td>
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<tr>
<td>increases with age</td>
<td></td>
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<tr>
<td>B. Homocysteine levels</td>
<td>Men &gt; age-matched women</td>
</tr>
<tr>
<td>men</td>
<td></td>
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<tr>
<td>III. Renal function</td>
<td>Homocysteine increases with increased creatinine</td>
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<tr>
<td>IV. Nutrition</td>
<td></td>
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<tr>
<td>A. Vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>Deficiency</td>
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<tr>
<td>deficiency</td>
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<tr>
<td>B. Vitamin B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>Deficiency</td>
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<tr>
<td>C. Folate deficiency</td>
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<tr>
<td>D. Disease states</td>
<td></td>
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<tr>
<td>A. Severe psoriasis</td>
<td>Increased homocysteine levels (possibly related to lower folate levels)</td>
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<td>associated</td>
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<tr>
<td>B. Cancer, acute lymphoblastic leukemia, elevated levels</td>
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<tr>
<td>C. Chronic renal failure</td>
<td>Increased homocysteine, lowered with dialysis</td>
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<td>VI. Medications</td>
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<tr>
<td>A. Increase homocysteine</td>
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<tr>
<td>methotrexate, depletes</td>
<td></td>
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<tr>
<td>methylenetetrahydrofolate</td>
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<tr>
<td>B. Acrithine, vitamin B&lt;sub&gt;6&lt;/sub&gt; antagonist</td>
<td></td>
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<tr>
<td>C. Nitrous oxide, inactivates vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
<td></td>
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<tr>
<td>D. Phenytoin, interferes with folate metabolism</td>
<td></td>
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<tr>
<td>E. Carbamazepine, interferes with folate metabolism</td>
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<tr>
<td>F. Estrogen-containing oral contraceptives, induce vitamin B&lt;sub&gt;12&lt;/sub&gt; deficiency</td>
<td></td>
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<tr>
<td>B. Decrease homocysteine: penicillamine, metabolically stable cyssteine analogue</td>
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Disease states. Psoriasis. Severe psoriasis is associated with elevated fasting plasma homocysteine levels. In one study, these patients had folate levels that, although normal, were lower than those of control subjects (4).

Cancer. Markedly elevated homocysteine levels have been seen in acute lymphoblastic leukemia and decrease after treatment with cytotoxic drugs (51). Moderately elevated homocysteine concentrations have also been seen in patients with various carcinomas, including breast, ovarian and pancreatic, who had highly elevated tumor markers. Serial samples from many of these patients demonstrated parallel changes in homocysteine concentration and tumor marker levels (52). Methionine metabolism may be altered in malignant cells: Most transformed cells, in contrast to nontransformed cells, are methionine dependent in culture and are unable to utilize homocysteine in media (53). Plasma levels may be related to the large burden of proliferating cells unable to utilize endogenous homocysteine.

Drugs. Plasma homocysteine levels may also be influenced by pharmacologic agents, including methotrexate, nitrous oxide, phenytoin, carbamazepine, azathiprine, estrogen-containing oral contraceptives and panicillamine (4.53). Methotrexate depletes 5-methyltetrahydrofolate, the cosubstrate for methionine synthase, and induces a transient increase in plasma homocysteine (4.53). Nitrous oxide inactivates vitamin B_{12} dependent methionine synthase and is known to elevate homocysteine (4). Anticonvulsants, such as phenytoin and carbamazepine, which interfere with folate metabolism, may also increase homocysteine concentrations (4.53).

Azathiprine, initially used for refractory cases of psoriasis, is a vitamin B_{6} antagonist and inhibits cystathionine beta-synthase, raising homocysteine levels. In 1976, azathioprine was prohibited by the Food and Drug Administration because it was associated with increased risk of thromboembolism (4). The use of diuretic drugs (54) and the lipid-lowering agents colestipol and niacin in combination (55) have been associated with elevated homocysteine levels.

Estrogen-containing oral contraceptives may alter the metabolism of sulfur-containing amino acids, including homocysteine (53). Although women taking oral contraceptives usually have reduced plasma homocysteine, high levels may be seen in some (56). A recent prospective study by van der Meer et al. (57) demonstrated a 10.9% decrease in fasting homocysteine levels in postmenopausal women treated with continuous micronized 17-beta-estradiol combined with cyclic dydrogesterone compared with baseline, pre-hormone replacement levels. The decrease in homocysteine levels was greatest for women with high baseline concentrations (a 16.9% decrease), and no significant changes in homocysteine concentration occurred in those with low homocysteine levels. These changes occurred over the first 6 months of therapy, after which no further decrease was found.

Panecillamine, a cysteine analogue with chelating properties used in Wilson's disease, heavy metal poisoning, rheumatoid arthritis and cystinuria, may lower homocysteine levels (53), possibly by the formation of a low molecular weight mixed disulfide. This reduction may be seen in patients with homocystinuria and even in those with rheumatoid arthritis with normal homocysteine levels before therapy.

Homocystinuria

Homocystinuria is a rare autosomal recessive disease characterized by markedly elevated homocysteine concentrations in the blood accompanied by passage of homocysteine in the urine (58). It is most commonly caused by a deficiency of cystathionine beta-synthase. Typical clinical manifestations include mental retardation, skeletal abnormalities and lens dislocations. There is also a marked tendency to both arterial and venous thrombotic episodes, with a 30% risk by 29 years of age (59,60). The frequency and extensive nature of vascular disorders in these patients has led to extensive work on the epidemiologic, clinical and pathophysiologic link between homocysteine and vascular disease. A review of the vascular pathologic and pathophysiologic mechanisms may provide useful insights into possible mechanisms of vascular disease in patients with elevated homocysteine levels.

Vascular pathology. Premature atherosclerosis occurs in patients with homocystinuria involving large, medium and small arteries and may affect any vascular bed. There is marked intimal thickening as well as medial muscular fiber splitting and fraying, with an increase in intersitial collagen. There may also be changes in the internal elastic lamina. The lesions are associated with proliferation of perivascular connective tissues containing increased number of fibroblasts, collagen bundles and small elastic fibers (27).

Pathophysiologic Mechanisms of Vascular Disease

Both clinical and experimental studies suggest that abnormally high homocysteine concentrations may be responsible for the atherogenic and thrombotic tendencies of homocystinuric and hyperhomocysteinemic patients, although the exact mechanism has not been fully elucidated.

Effects on endothelium. In vitro studies. Several studies have examined the direct cytotoxic effects of homocysteine on endothelial cells grown in tissue culture (61,62). Homocysteine thiolactone produced concentration-dependent endothelial cell damage that was prevented by catalase, suggesting an important role for hydrogen peroxide in the pathologic process (63). Starkebaum and Harlan (62) found that copper led to the oxidation of homocysteine, producing hydrogen peroxide. Cultured endothelial cells exposed to homocysteine were lysed in a time- and dose-dependent manner, but only with added copper (62). These findings may be relevant to patients with homocystinuria, in whom exceptionally high circulating homocysteine and elevated copper concentrations (64) may be seen. The significance in those with less striking elevations of homocysteine is unclear, especially because cop-
per concentrations have been reported to be lower in patients with coronary artery disease than in control subjects (65).

Because prostacyclin is a potent inhibitor of platelets, reduced synthesis of this substance could predispose to thrombosis. However, there are conflicting results on the effects of homocysteine on the synthesis of prostacyclin in cultured cells (66,67). One study suggests (68) that endothelium-derived relaxing factor may be protective against the effects of homocysteine, a protection that may be lost with progressive endothelial damage. Recently, Tsai et al. (69) reported a growth-promoting effect of homocysteine on smooth muscle cells and a decrease in endothelial cell DNA synthesis.

In vivo studies. Harker et al. (70) found that infusion of \( l \)-homocysteine for 5 days into baboons caused patchy desquamation of vascular endothelium as well as a decreased platelet survival time. They suggested that arterial thrombus formation in homocystinuric patients resulted from sustained homocysteine-induced endothelial injury with secondary increase in platelet consumption and atherogenesis. To examine the long-term effect of such infusions, a 3-month study was repeated in baboons (71,72). Endothelial damage increased with the concentration of homocysteine. In these studies, antiplatelet agents were administered, which prevented some of the adverse effects. Conflicting results have been seen in other animal studies using pigs (73,74), rabbits (75,76), and monkeys (77,78). The reproducible findings of inducible vascular damage in the baboon (70–72), but not in other animals, suggest that important species-dependent responses may be involved. In a recent study of minipigs fed a methionine-rich diet, high homocysteine concentrations were seen after 4 months on this diet, and 2 of the 10 animals developed thromboemboli (79). In addition, pathologic changes in the elastic lamina were noted in these animals. Human studies using flow-mediated dilation have demonstrated abnormal endothelial function in homocystinuric patients but not in their heterozygote parents (80).

An additional confounding issue in many studies has been the use of different forms of homocysteine, especially homocysteine thiolactone (75,76). Homocysteine thiolactone may be more cytotoxic to endothelial cells than homocysteine alone, possibly because of an independent toxic effect of thiolactone. Furthermore, because the naturally occurring form of homocysteine is the \( l \)-isomer, using the unnatural \( d \) isomer (72) may further complicate the matter.

Effects on platelets. In addition to a direct pathologic effect on endothelial cells, a role for the effect of homocysteine on platelets has also been suggested. Survival of platelets has been shown to be impaired in baboons after administration of homocysteine and in four patients with cystathionine beta-synthase deficiency (70,71). However, in other patients with homocystinuria, platelet survival has been normal (81,82).

Increased adhesiveness of platelets was first shown by McDonald et al. (83), who added homocysteine to normal blood to increase the concentration to that found in cystathionine beta-synthase-deficient patients, even though this was not found by others (70,81,84). Different patient populations, methodologies or measurements of platelet variables may explain some of the differences.

Abnormalities in adenosine diphosphate–induced platelet aggregation have been seen in some homocystinuric patients but not in others (60). Grabeck et al. (85) showed that in vitro \( l \)–homocysteine alters arachidonic acid metabolism of normal platelets so that 12-hydroxy-5,8,10-heptadecatrienoic acid and thromboxane \( A_2 \) are increased. Recently, elevated thromboxane \( A_2 \) formation was reported in homocystinuric patients (86). Homocysteine induced alterations in arachidonic acid metabolism, resulting in the accumulation of powerful platelet aggregators in patients with vascular episodes, could provide a possible mechanism for thrombosis.

Effects on clotting factors. Homocysteine may also affect many factors involved in the clotting cascade. Gianulli et al. (87) reported reduced serum antithrombin activity in seven homocystinuric patients compared with that in normal age- and gender-matched control subjects. Similar findings have been reported by others (4,88). Levels of antithrombin activity 50% of normal have been associated with an increased tendency to intravascular thrombosis (89). Curiously, Palareti et al. (88) normalized the antithrombin activity using pyridoxine and folic acid, but circulating homocysteine levels remained high so that reduced antithrombin concentrations may not be related to the elevation in homocysteine (88). A decreased level of Factor V (84) and reduced Factor VII concentrations have been seen in some studies (4,60).

Several in vitro studies have shown that homocysteine promotes procoagulant activity (90,96). Rodgers and Kane (90) found that homocysteine activated Factor V in a dose-dependent manner and also increased prothrombin activation of Factor Xa. In addition, homocysteine inhibited protein C activation in cultured endothelial cells (91). Two studies have demonstrated that homocysteine stimulates thrombomodulin mRNA levels in endothelial cells (92,93) but may block its passage through the secretory pathway, resulting in the inhibition of cell surface expression (92). Secretion of plasminogen activator inhibitor I was not affected by homocysteine (92). Homocysteine also inhibits the cofactor activity of cell surface thrombomodulin (93). Antithrombin III binding and von Willebrand factor secretion may also be affected by homocysteine (94,95). Hajjar (96) found that homocysteine blocked the binding of tissue-type plasminogen activator when added to human endothelial cells. These observations suggest that homocysteine promotes prothrombotic activities in the vascular endothelium. Additionally, a correlation between homocysteine and fibrinogen has been reported in a study of patients with coronary artery disease (97).

Hyperhomocysteinemia and Vascular Disease

Vascular complications in patients with homocystinuria have been well known since early descriptions of the disease. However, it was not until 1976 that the first controlled study showed a clear difference in methionine metabolism and homocysteine concentrations between patients with premature
vascular disease and normal control subjects (11). To date, many studies (12-15,17,19-22,26,41,49,54,98-106) involving several thousands of patients have shown higher plasma homocysteine levels in patients with vascular disease than in normal subjects. The methodologies of these different studies have varied widely not just in the technique of evaluation and definition of hyperhomocysteinemia but also in sample sizes, types of patients and control subjects, evaluation of other risk factors, statistical techniques, types of vascular disease and definitions of end points. Despite their differences, the studies have similar conclusions.

Milestone studies of homocysteine and coronary atherosclerosis. Patients with coronary disease were first investigated by Wilcken and Wilcken (11), who studied methionine metabolism in patients <50 years old with angiographically proven coronary artery disease. Using the methionine loading test, they found higher postload homocysteine mixed disulfide in patients than in control subjects. In 1983, this group undertook another study of patients with coronary artery disease but were unable to confirm their previous findings (24).

Boers et al. (12) examined patients with vascular disease, including some with coronary artery disease. Using a methionine loading test, they found significantly higher levels of homocysteine in their patients with peripheral vascular disease and stroke but not in those with coronary disease. Activity of cystathionine beta-synthase was low in patients with stroke and peripheral vascular disease, suggesting heterozygosity for homocystinuria. This was not seen in the patients with coronary artery disease. It should be emphasized that the numbers in this study were small, and most studies have since confirmed an association between coronary artery disease and high circulating homocysteine concentrations. In 1991, Clarke et al. (13) compared homocysteine levels after methionine loading in patients with premature vascular disease (<55 years old) to those in normal subjects. Hyperhomocysteinemia was seen in 42% of patients with cerebrovascular disease, 28% with peripheral vascular disease and 30% with coronary artery disease but in none of the control subjects. After controlling for conventional risk factors, the odds ratio for vascular disease in patients with hyperhomocysteinemia was 3.3. An odds ratio higher than that for smoking or hypercholesterolemia. Approximately 80% of patients with high homocysteine concentrations had low cystathionine beta-synthase activity. Clarke et al. concluded that a partial deficiency of cystathionine beta-synthase was the most frequent cause of hyperhomocysteinemia even though vitamin B<sub>12</sub> and folate levels were lower in patients with high homocysteine concentrations.

Because of the conflicting results that had been obtained in some of the earlier investigations and a lack of standardized methodology, a multicenter case-control study was undertaken in Europe in 1990 (107). With methodology and definitions of vascular disease determined by consensus, ~800 patients and 800 control subjects were studied. That study confirmed the independent association of high homocysteine concentrations with all forms of premature vascular disease (105). The large sample size will enable further evaluation of possible risk factor interactions.

In 1992, high homocysteine concentrations were reported to be an independent risk factor for myocardial infarction in male participants prospectively enrolled in the U.S. Physicians Health Study (103). A threshold for increased risk of disease was noted at a homocysteine concentration of 15.8 nmol/ml, corresponding to the 95th percentile for control subjects.

A similar study was reported from Norway (102). Homocysteine concentrations were higher in patients who subsequently had a myocardial infarction than in those who did not. Unlike the study of Stampfer et al. (103), however, no threshold phenomenon was observed.

More recently, another study from Finland (108) found no relation between coronary disease and homocysteine in a group of 7424 men and women followed up for 9 years. Control subjects were selected for patients who subsequently had a myocardial infarction or stroke. Total homocysteine concentration was measured in serum. Similar mean values were seen in patients and in control subjects (~10 μmol/liter). Because serum values are usually higher than those for plasma (16), the findings suggest that exceptionally low plasma values are seen in this area in Finland. Although the investigators attribute this to a low gene frequency for cystathionine beta-synthase deficiency, the technique of measurement of homocysteine may have accounted for the relatively low values. Furthermore, data were not reported for serum concentrations of B group vitamins in this population, which are of major importance in regulating plasma homocysteine.

In 1991, Kang et al. (34) explored the role of the enzyme methylenetetrahydrofolate reductase in patients with coronary artery disease and found an abnormal thermolabile variant of this enzyme. In that study, but not in another (35), this abnormality was associated with high homocysteine concentrations. These studies have highlighted the possible importance of enzyme abnormalities other than cystathionine beta-synthase in the genesis of high homocysteine concentrations.

Only recently has the relationship between homocysteine and concentrations of vitamin B<sub>12</sub>, folate and vitamin B<sub>6</sub> been explored in patients with coronary artery disease. Both folic acid and vitamin B<sub>12</sub> were reported to be lower in patients with higher homocysteine concentrations (13,19). Negative correlations were found between plasma homocysteine and both serum vitamin B<sub>12</sub> and folate (41,49) in patients with coronary artery disease. At the Cleveland Clinic Foundation, significant negative correlations both between folate and homocysteine and between vitamin B<sub>12</sub> and homocysteine have been seen in patients with coronary artery disease as well as in normal subjects (16,109). An additional finding of a negative correlation between homocysteine and vitamin B<sub>6</sub> in that study concurs with others (41). Frank vitamin B<sub>6</sub> deficiency was seen in 10% of patients compared with only 2% of normal subjects. These findings are consistent with earlier in vivo work in which arteriosclerotic lesions had developed in animals chronically deprived of vitamin B<sub>6</sub> (77,78). Such studies have shifted the emphasis from a genetic etiology for hyperhomocysteinemia...
and have highlighted the importance of a possible role of lower vitamin levels in the development of vascular disease, including coronary artery disease. However, the precise interrelation between nutritional and hereditary factors still requires further evaluation.

**Hyperhomocysteinemia and conventional risk factors in cardiovascular disease.** Several studies have shown no relation between plasma homocysteine and serum cholesterol (14, 17, 22, 26, 100). In a recent study by Mølgaard et al. (101), plasma homocysteine correlated with both low density lipoprotein (LDL) cholesterol and apolipoprotein B levels, but these relations resulted from covariation with serum folate and could not be confirmed with multiple linear regression analysis. Kang et al. (15) demonstrated a correlation between plasma homocysteine and cholesterol in patients in whom the level of cholesterol was in the normal range, and Wu et al. (41) showed a correlation with LDL cholesterol.

Most studies have shown that smoking is not linked with homocysteine in patients with vascular disease (14, 15, 22, 41, 98, 100, 101), although a few studies have shown such a correlation (25, 110, 111). No relation between blood pressure and homocysteine concentration has been seen in most studies of patients with coronary artery disease (14, 15, 20, 22, 41, 49, 54, 98, 100). However, Malinow et al. (17) found that 77% of patients with elevated homocysteine concentrations were hypertensive compared with 40% of those with normal levels. In another study (38), total plasma homocysteine was significantly elevated in hypertensive patients compared with that in normotensive subjects. Homocysteine also correlated with serum creatinine, but, even after adjusting for this, the relation between homocysteine and hypertension persisted. Several studies have shown no correlation between diabetes or blood sugar and hyperhomocysteinemia (14, 17, 22, 41, 98, 99, 101).

**Summary.** Hyperhomocysteinemia is an independent risk factor for vascular disease, including coronary atherosclerosis. Possible interactions with or modifications of the effects of other risk factors are suggested by some studies that have shown interactions with LDL fractions (41, 112, 113). Glueck et al. (110) showed that the risk of a myocardial infarction in a group of hyperlipidemic patients was greatest in patients with hyperhomocysteinemia and low high density lipoprotein, raising the possibility of a clinically important interaction between these risk factors.

**Therapy**

Therapeutic regimens for the reduction of homocysteine in patients with homocystinuria have been extensively reviewed elsewhere (4, 60). Elevated plasma homocysteine levels may also be reduced in conditions other than homocystinuria as well as in normal subjects (48, 114, 115). Homocysteine levels in folate-deficient patients can be lowered by oral folic acid replacement (46). In one study, the elevated homocysteine concentrations in patients with renal failure decreased after only 2 weeks of folic acid therapy (116). Maximal effects may be seen after 4 (116) to 6 (48) weeks of therapy. The lowest effective dose for folic acid supplementation has not yet been determined. Doses of 10 mg (26) or 5 mg alone (114) or 1 mg in conjunction with vitamins B12 and B6 may be effective (48). Recently, Ubbink et al. (115) demonstrated the efficacy of a dose as low as 0.65 mg folic acid, which lowered plasma homocysteine concentrations in patients with high levels by 45%. Brattström et al. (117) noted significantly lower homocysteine concentrations in middle-aged and elderly subjects taking multivitamins containing doses of folic acid ranging from only 200 to 400 μg. Homocysteine values increase if vitamin therapy is discontinued (116). In one study, homocysteine concentrations returned to pretreatment levels within 10 weeks of cessation of folic acid therapy in renal transplant recipients (116).

Because vitamin B12 is a cofactor in remethylation and not a substrate, B12 therapy would not be expected to reduce homocysteine unless there is suboptimal B12 status or frank B12 deficiency. Results of a limited number of studies support these expectations (114). Administration of vitamin B6 alone in normal subjects does not lower fasting plasma homocysteine concentration (114, 115).

**Therapeutic studies in patients with vascular disease.** Several studies have been performed in patients with peripheral and cerebrovascular disease. In one study, folic acid combined with vitamin B6 reduced fasting homocysteine levels by 53% (26). However, vitamin B6, when administered alone, had no effect on fasting homocysteine concentrations but may lower the levels after a methionine load (26). Similar observations have been made by others (12, 104, 118).

These findings have prompted studies of folic acid and vitamins B6 and B12 in patients with coronary atherosclerosis to reduce homocysteine concentrations (104, 119–121). Lindgren et al. (121) have recently successfully used doses of 2.5 and 10 mg of folic acid to reduce homocysteine concentrations in a small group of patients after myocardial infarction. Dudman et al. (104) lowered homocysteine concentrations in patients with coronary disease using a variety of treatment regimens, including folic acid, vitamin B6, choline and betaine. Ryan et al. (119), using a combination of vitamin B6 and folic acid, observed a decrease in postload plasma homocysteine concentration by 15%. In a recent study, Saltzman et al. (120) used placebo, folic acid, vitamins B12 and B6, together, or a combination of all three vitamins in patients undergoing coronary angioplasty (120). Folic acid alone, or combined with vitamins B12 and B6, or the combination of B12 and B6 alone, each reduced homocysteine concentrations. Recently, Naurath et al. (122) used an intramuscular regimen of 1.1 mg of folate, 1 mg of vitamin B12, and 5 mg of B6 in a wide variety of patients, including those with vascular disease, and reduced 80% of elevated homocysteine levels to the normal range. In preliminary studies at the Cleveland Clinic, 400 μg of folic acid combined with vitamins B12 and B6 reduced plasma homocysteine concentrations by 15% in patients with coronary disease. However, ~35% of patients showed no significant response. The relatively small change in homocysteine concentration produced by this treatment regimen and the relatively large
percentage of nonresponders suggest that a dose >400 \( \mu \)g of folate should be used. The optimal dose and vitamin combination to produce the maximal reduction of homocysteine in coronary artery disease has not yet been established, nor has the effect of lowering homocysteine concentration on cardiovascular risk.

**Emerging Aspects of Homocysteine and Coronary Disease**

**Effects of gender, age and race.** Although high circulating homocysteine concentrations are associated with coronary artery disease, most previous studies have focused on younger male patient populations. However, in an earlier study, Kang et al. (15) pointed to elevated homocysteine concentrations in women with coronary disease, although their precise risk of disease with hyperhomocysteinemia remained unclear. At this institution, an odds ratio for coronary artery disease in women age 35 (109) was found, showing that there is an increased risk of coronary disease in women as well as in men. We have also noted odds ratios for coronary disease of 3.2 in both male and female patients >65 years old compared with 2.9 <65 years old, suggesting that high homocysteine concentrations may also be an important risk factor in the elderly (109). Recently, McMartin et al. (123) examined the effect of race and gender on homocysteine concentrations in patients with vascular disease. They found that white men had the highest levels (14.0 \( \mu \)mol/liter), which were significantly higher than those in white women (8.5 \( \mu \)mol/liter) or black men (10.0 \( \mu \)mol/liter). There was no significant difference between black men and women (10.0 \( \mu \)mol/liter vs. 10.4 \( \mu \)mol/liter). These preliminary data suggest a possible difference in the prevalence and impact of hyperhomocysteinemia on vascular disease between races, though this study included only a small number of patients. Additionally, ages and vitamin levels were not reported. Further studies are needed to elucidate the impact of race on homocysteine levels and its relative risk.

**Severity of coronary artery disease.** Increasing severity of carotid disease with increasing homocysteine levels has been seen (106,124), but reports on the relation between severity of coronary disease and homocysteine concentration are conflicting. Kang et al. (15) reported no correlation between homocysteine concentrations and Gensini scores of severity of coronary disease. However, Uthiker et al. (21) did show a correlation between homocysteine levels and the number of occluded coronary arteries, consistent with the findings of others (97). Using intravascular ultrasound, we observed less intracoronary plaque in “normal” reference segments in patients undergoing coronary interventions with plasma homocysteine <11 \( \mu \)mol/liter compared with those with higher concentrations (125). In addition, the patients with lower homocysteine concentrations had fewer adverse clinical outcomes after coronary intervention. The precise relation between homocysteine and the severity and distribution of coronary disease requires further study.

**Cardiac transplantation.** Coronary atherosclerosis is a major cause of late morbidity and mortality in cardiac transplant recipients. After transplantation, homocysteine concentrations have been found to increase (126,127). Berger et al. (127) recently reported a study of 44 recipients in whom homocysteine levels increased 70% after heart transplantation, whereas \( B_12 \) and folate levels decreased by 49% and 20%, respectively. Glomerular filtration rate also decreased 25% (127). Levels in transplant recipients studied at the Cleveland Clinic Foundation were 18.5 \( \mu \)mol/liter but were only 11.0 \( \mu \)mol/liter in age- and gender-matched control subjects. Deficiencies of both folate and vitamin \( B_12 \) (pyridoxal 5'-phosphate <20 \( \mu \)mol/liter) were more common in the transplant group, demonstrating the presence of widespread nutrient disorders in these patients. However, renal function was also impaired, possibly from toxicity of the immunosuppressive agents. Mechanisms of these alterations in homocysteine metabolism require further evaluation, but the abnormalities of \( B_12 \)-folate and renal function suggest a complex multifactorial etiology.

**Conclusions**

Prospective and case-control studies have shown that modestly elevated plasma homocysteine concentration is a risk factor for coronary artery disease. Elevations in homocysteine may be caused by a combination of nutritional and genetic factors. The exact mechanism for the development of atherosclerosis in this setting is unclear. Folic acid may be used to reduce homocysteine concentration in patients with coronary artery disease, either alone or combined with other \( B \) vitamins. The optimal doses and combinations of these substances remain unclear and are currently under investigation. The possible beneficial effect of such treatment on coronary risk, including effects on cardiac morbidity and mortality, will require a long-term, prospective randomized placebo-controlled trial.

**References**

7. Svendsen AM, Djurhuus R, Refsum H, Ueland PM. Disposition of homocysteine in rat hepatocytes and in nontransformed and malignant mouse


113. Pannascharatny S. Oxidation of low-density lipoprotein by thiols compounds leads to its recognition by the acetyl LDL receptor. Biochim Biophys Acta 1987;917:337–40.